

THE USE OF SILICONE RUBBER PLASTIC FOR REPLICATING LEAF SURFACES

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SUMMARY

The numbers of open and closed leaf stomata were investigated both in *Plantago major* and in *Epilobium angustifolium* in relation to, respectively, the relative humidity of the surrounding air, the transpiration and the evaporation, by studying transparent replicas of the leaves obtained by using the replica method, described by SAMPSON (1961). Statistical operations with the data obtained showed in both cases a significantly lower number of open stomata when the relative humidity is lower and transpiration is higher.

1. INTRODUCTION

In the course of ecological studies on some plant species it appeared necessary to obtain some information on the manner in which both the numbers and the aperture of their leaf stomata are related to various factors such as relative humidity of the air, leaf transpiration and the evaporation of a free water surface. As the method to be used had to be applicable both in greenhouse experiments and in the field and we were committed to our objects, our possibilities were practically restricted to some variant of the replica method.

HARTSUIJKER (1935), WENZL (1939) and many others, critically reviewing various methods of measuring the aperture of leaf stomata and carrying on their own experiments, think it hardly possible to overcome the difficulty that the stomatal aperture to be measured is in some way influenced either by the applied substances or by the whole procedure, which is necessary to solve the problem. It is true that the objections do not count equally heavily for the leaves of different plants, but there is a general agreement that one has to proceed with caution when studying the results of experiments carried out using this method.

However, in 1961 SAMPSON reported success in obviating these difficulties. She replicated both moist and dry surfaces by making use of a mixture of a non-toxic silicone rubber monomer and a catalyst by which the rubber is rapidly hardened, so obtaining a primary negative replica of, among others, leaf surfaces. A secondary, positive, transparent impression of this replica was then made by making use of a clear nail polish. After being mounted between two thin microscope slides the second replica was ready for examination under the microscope. It appeared to reveal the smallest details of all the features of the original surface and since the rubber and the catalyst appeared to be non-toxic,

serial impressions could be made from the same regions provided the surface is not damaged when the rubber is removed.

The method was also applied by ZELITCH (1961) and by SHUTAK & DAYAWOM (1966) in their experiments on the opening and closure of leaf stomata. They, too, concluded that the silicone rubber plastic was non-toxic. The same leaves could be used several times.

IDLE (1969) made use of this method to obtain replicas for examining leaf surfaces under the Scanning electron microscope.

2. MATERIAL AND METHOD

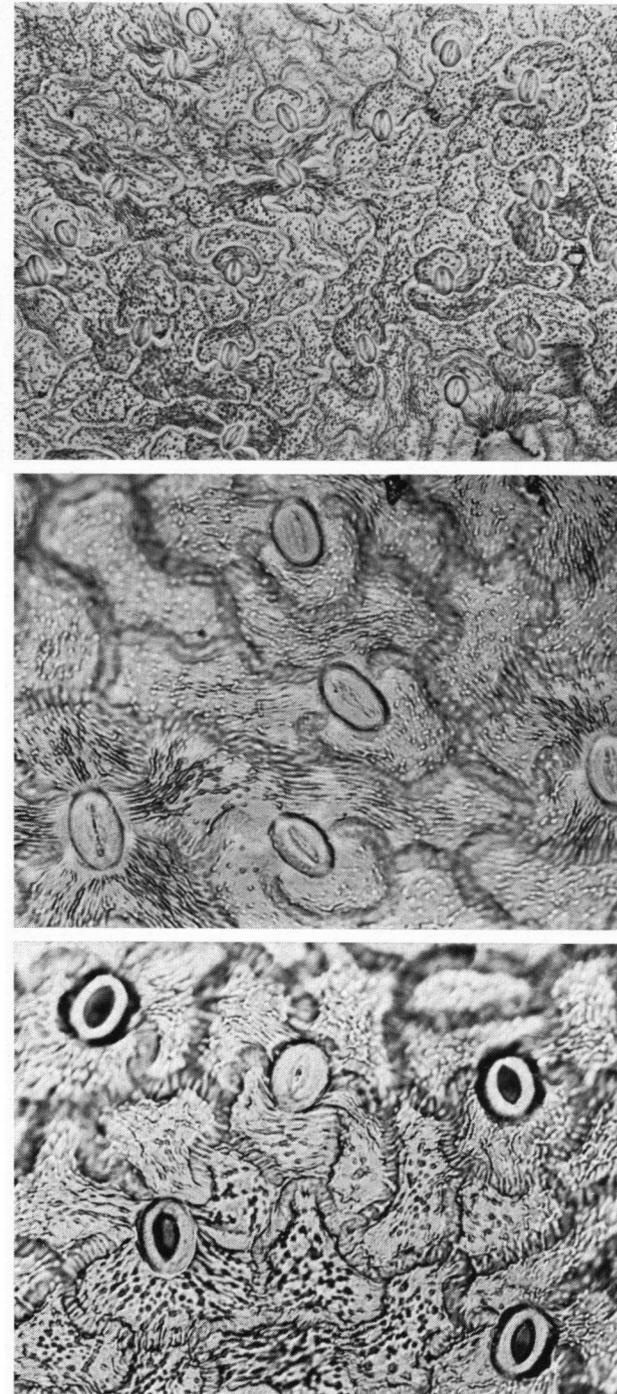
The usefulness of the method described by SAMPSON (1961) was tested in two species a. The leaf stomata of two months old plants of *Plantago major* L., grown in the greenhouse at temperatures of about 20°C and a relative humidity of about 70%. During the first month the plants were grown in gravel provided with a nutrient solution; during the second month they were cultivated in small plastic pots filled with ordinary garden soil, each containing one plant. Two hours before a replica of the leaf surfaces was made the plants were placed in boxes, each provided with a sluice. The relative humidity in the first box was kept at 30% (25–40%) and in the second box at 97% (95–100%), other environmental conditions such as light intensity and temperature being equal though not constant. The boxes were fitted with a glass wall; additional illumination was given by strip lighting.

b. The leaf stomata of adult plants of *Epilobium angustifolium* L., occurring either at the northern or at the southern slope of a dune at Schiermonnikoog, one of the Dutch Frisian islands.

In both experiments silicone rubber plastic (R.T.V.-11, Silicone Products Department, General Electric Company, Waterford, N.Y., U.S.A.) was mixed with a few drops of a catalyst (Silicure T-773) and immediately afterwards spread over the leaf surface to be examined with a wooden spatula. The mixture hardens in less than two minutes and can then be removed from the leaf with forceps. The replicas obtained in this way were carefully washed in distilled water to which a small quantity of the detergent "Teepol" was added and washed again in pure distilled water. After blotting these replicas with fluff-free filter-paper they were placed in a desiccator containing phosphorus pentoxide or in a dustfree incubator at 35°C. After thoroughly drying the replicas were coated with a clear nail polish without etheric oils in a dustfree environment. The nail polish ("My lady" brand) could easily be obtained in the well-known Hema-shops in Amsterdam. Half an hour later the nail polish was quite hard and could easily be stripped off from the rubber. Thus a secondary transparent replica was obtained that was mounted on a dry microscope slide and covered by a cover-glass. To prevent the penetration of dust and water vapour the cover-glass was sealed with nail polish or Depex.

Observation in the light microscope showed that the secondary replicas possess all the characteristics of the leaf surfaces from which the primary replicas

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Replicas of leaf surfaces of *Plantago major*

(replicas by Mr. N. van Strien)

Fig. 1. Closed stomata. $\times 200$

Fig. 2. Closed stomata. $\times 500$

Fig. 3. Open stomata. $\times 500$

were taken and it appeared easy to distinguish both the open and the closed stomata and to count their respective numbers (*figs. 1, 2 and 3*). As the hardening of the silicone rubber plastic after mixing it with the catalyst took less than two minutes it seems reasonable to assume that the transparent replicas furnished information on the real situation of the leaf surfaces with regard to stomatal opening at the beginning of the experiment.

In the experiment with *Plantago major* the numbers of the open and closed leaf stomata were related to the different relative humidities of the surrounding air; in that with *Epilobium angustifolium* to both transpiration and evaporation at the north, respectively the south slope of the dune.

3. RESULTS

The results of experiments in which 51 leaves of the two months old plants of *Plantago major* were replicated are given in *table 1*. The observations in plants placed in air with a relative humidity of 30% cover 24 leaves, those in plants placed in air with a relative humidity of 97% cover 27 leaves.

Table 1. Number of stomata per square centimeter and percentage of open leaf stomata in *Plantago major* under different conditions of relative humidity

Relative humidity %	Total number of stomata per cm ²	Percentage of open stomata % of total
30 (25– 40)	679 (333– 899)	16.6 (1.3–38.3)
97 (95–100)	670 (421–1062)	24.2 (4.5–47.8)

It is to be expected that the total number of stomata proved to be equal in both cases as the plants were grown in greenhouses under the same environmental conditions and were placed under different relative humidities only some hours before the replicas were made.

The data on the percentage of open and closed stomata were statistically treated by a Chi-square test; it appeared that the difference between the two groups of plants is significant. The percentage of open stomata is higher under conditions of high humidity.

In the experiment with *Epilobium angustifolium* the data were obtained by counting the replicas of 128 leaves, 64 from plants at the north slope and 64 from plants at the south slope. The results of these countings are given in *table 2*.

There was no difference between the plants from the north and the south slope of the dune in the number of leaf stomata per cm². Statistical operations with the data on the numbers of open and closed stomata showed that there are significantly more open leaf stomata in plants growing at the north slope compared with those growing at the south slope. Both the transpiration and the evaporation appeared to show a significantly lower value at the north slope

Table 2. Relation between transpiration/evaporation and the number of open leaf stomata in *Epilobium angustifolium* both at the north and the south slope of a dune at Schiermonnikoog.

	North slope		South slope	
Number of stomata (per cm ²)	733	(372-1177)	744	(355-1408)
Percentage of open stomata	84.0	(47.2-98.4)	80.9	(38.0-96.1)
Transpiration (mg/cm ² .min.)	0.247	(0.065-0.800)	0.285	(0.063-0.774)
Evaporation (mg/cm ² .min.)	0.227	(0.105-0.540)	0.313	(0.127-0.445)

than at the southern side of the dune. Apparently the north slope is more humid than the south slope. Comparing these results with those given in *table 1* there appears to be a striking agreement: in both cases there is a positive correlation between the percentage of open stomata and the humidity of the surrounding air.

4. DISCUSSION

From the preceding paragraphs it can be concluded that the replicating method as described by SAMPSON is valuable to obtain data to which it is possible to apply statistical operations in order to answer the question whether an observed difference is significant or not. The method can easily be applied in the field. Other advantages of the method: (1) it can be used both on dry and moist surfaces; (2) it is not necessary to use detached leaves, and (3) each leaf can be used over and over again.

For ecological field work such a method may be of great importance as many methods for obtaining data on the aperture of leaf stomata are far too complicated for application under field circumstances.

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